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**Contact: Prof. Bassam Z. Shakhashiri**

**UW-Madison Department of Chemistry**

**bassam@chem.wisc.edu**

**www.scifun.org**

Disrupting the Balance: On the Host-Pathogen Homeostasis of *M. tuberculosis* from vaccine characterization to superinfection dynamics

by

Rachel Erickson Hildebrand

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The dissertation is approved by the following members of the final oral committee:

Adel. M. Talaat, Professor, Pathobiological Sciences, Advisor

Caitlin Pepperell, Professor, Medical Microbiology and Immunology

Wilmara Salgado-Pabón, Professor, Pathobiological Sciences

Garret Suen, Professor, Bacteriology

Marulasiddappa Suresh, Professor, Pathobiological Sciences

# CHAPTER SIX

## Chapter for the Public

**For the Reader**

I wrote this chapter because I believe that science should be accessible to everyone and it is an investment the scientific community needs to commit continually to making. Programs that I participated in, such as the life sciences communication minor and WISCIENCE Public Service Fellows, have only reaffirmed this belief. A thank you to the WISL program that aids students in completing a chapter for the public, without which I likely would not have considered writing this.

**What is Homeostasis?**

Homeostasis is a scientific word that describes how living organisms constantly struggle to maintain balance. This is not balance in terms of 'can you stand on one leg' but an internal balance like temperature or nutrients. Being out of balance can have deadly effects. For example, the human body has many ways to try and maintain its internal balance in order to survive. We ideally maintain an internal temperature of around 98°F. When it's hot outside, we sweat in an effort to cool down (1). Our skin may also flush as blood rushes closer to the surface where it can lose heat more easily. When it's cold, we shiver- involuntary muscle twitching- causing our muscles to generate more heat (1). You, as a living organism, have many signals driving you to maintain an internal balance.

All living things practice homeostasis to survive. Just like you, other living organisms can get too hot or too cold. As we seek to understand other living organisms, we look at how they react to different environments in order to maintain their internal processes.

**Disease and Homeostasis**

Homeostasis can also refer to a biological system, not just a single organism. Our gut and its microbiome are a good example of this. In our gut, we have a variety of different bacteria

that, for the most part, do not harm us and in fact are beneficial (2). However, disruption to your body's internal processes can shake the homeostasis in this biological system. For example, certain gut conditions make oxygen more available to the different types of bacteria that reside there (3). This shift benefits some bacteria over others and can change the population of our gut. Bacteria that are usually helpful suddenly are causing disease (3). Here, homeostasis can turn from beneficial balance to sickness.

We usually think of disease in terms of 'sick' and 'well' with no separate state in between. And with many different illnesses, there is never a homeostasis where each organism survives for prolonged periods. Instead, it is a battle in which either we clear the organism from our system or the organism is able to keep growing and make us very sick. However, there are many examples of different pathogens who maintain a homeostasis between them and their host. Just like the example of the relationship between us and our gut microbiota, disturbances to the system can result in disease.

My thesis is about a pathogen whose default is homeostasis with the host population. *Mycobacterium tuberculosis*, or *M. tuberculosis* for short, infects one quarter of the human population (4). Humans are its only natural host- meaning outside of labs, only humans carry the disease. While *M. tuberculosis* infects so many, most of these infections never result in illness. *M. tuberculosis* can exist within a human for decades with no obvious adverse effects. In fact, only about 10% of people with *M. tuberculosis* ever get symptoms and spread the disease (5). This may seem counterintuitive- wouldn't it benefit a pathogen to sicken the maximum number of people and increase its own spread? The short answer is no- by only getting a percentage of the population it infects very sick, *M. tuberculosis*, which only infects humans, also ensures its

future (6). Too many illnesses and death could decrease its only host population and limit its spread long-term.

How did this come to be? Well, the association between humans and *M. tuberculosis* is estimated to go back around 70,000 years (7). This association has led to co-evolution which very simply means that as we changed, so did *M. tuberculosis*. The homeostasis that exists now between us is probably a reflection of this (6).

I don't want you to get the wrong idea here- the homeostasis humans have with *M. tuberculosis* is **not** like the mutually beneficial relationship we have with our gut microbiota. It is more like a stalemate in a war. Tuberculosis (TB) is a very serious disease. In the last decade, it has killed an average of 1.5 million people every year and, before antibiotics were available, it was responsible for 20% of all human deaths (4, 7). Additionally, the current vaccine for *M. tuberculosis* does not protect from the disease in adults (8). In short, it remains a very serious and very urgent problem in terms of global health. In my thesis, I use the lens that *M. tuberculosis* disease is a disruption of homeostasis as a way to improve our understanding of the pathogen with applications in treatment, vaccine development, and prevention.

**Examples of Homeostasis in *M. tuberculosis***

Simplistically, there are two types of outcomes with *M. tuberculosis* infection. Some people infected can have "active disease" where they have symptoms and spread the organism. Some people are able to control the infection resulting in "latent disease". In latency, the bacteria are still able to persist in the host but the host does not show symptoms. Treatment can take an active disease to a latent one. A latent disease can also progress to an active one- this is called reactivation. About 90% of *M. tuberculosis* infections are in the latent TB infection (LTBI) category (Fig. 1).

On the surface, LTBI can be invisible without any symptoms. Underneath the surface, there is a complex and highly organized process occurring to reach the stalemate between host and pathogen. Both the host and mycobacteria drive these processes. Here, I will focus on one particular outcome that is heavily associated with *M. tuberculosis* and can also serve as representative of the many processes leading to latency.

A granuloma is a type of tissue lesion, seen mostly in the lungs, made up of different kinds of immune cells. Once an *M. tuberculosis* infection occurs, a granuloma begins to develop around the area the bacterial cells initially infected (9). Gradually a large number of immune cells will surround the bacteria and infected human cells, creating a barrier that is thought to contain the mycobacteria to the lesion and prevent them from spreading (Figure 2). The granuloma also changes the environment the bacteria are in. Granulomas limit nutrients and oxygen to the bacteria, forcing them to change their diet and sometimes even go into a non-replicating state known as dormancy (10).

However, granulomas are not necessarily a good thing for the host. For one, they are a form of tissue damage. Secondly, although the bacteria remain entrapped inside, under certain conditions granulomas may break apart releasing the bacteria into surrounding tissues (9). Finally, there is plenty of evidence suggesting that the center of granulomas can serve as an area in which *M. tuberculosis* can live very easily (11).

As an *M. tuberculosis* infection becomes chronic, granulomas begin forming. *M. tuberculosis* must make changes to survive the new surroundings. *M. tuberculosis* utilizes gene expression to adapt to these changes. Gene expression occurs when information contained in DNA is made into proteins or other molecules (Figure 3a). The DNA is “read” by one protein making a transcript called mRNA. This transcript is then translated into a string of amino acids,

the building blocks of proteins, by a separate protein. Once done, the string of amino acids folds into the protein the cell uses. Making proteins requires resources, so genes are only expressed when needed. During the initial phase of infection, *M. tuberculosis* is focused on replicating and infecting new host cells. It expresses genes that help build cell walls for new cells and escape the host cells to infect new ones. Once the infection becomes more chronic, *M. tuberculosis* must adapt to the pressures of the granuloma such as hypoxia (low oxygen) and nutrient starvation. Instead of focusing on replicating, it expresses genes that help manage these new pressures and sometimes transition to a dormant, non-replicating state. Studying the genes that help *M. tuberculosis* survive these environments and persist for so long in humans can help us to develop new vaccines and treatments.

**Live-attenuated Vaccines and Dormancy Regulators**

One way to develop vaccines is to attenuate- or weaken- the pathogen itself. The vaccine then can cause a similar immune response as the pathogen, but is not strong enough to cause disease. These vaccines are known as live-attenuated vaccines. One real world example is *M. bovis* Bacillus Calmette-Guérin or BCG. This organism is related to *M. tuberculosis* but is usually unable to cause illness in the host. For *M. tuberculosis*, an ideal live-attenuated vaccine never reaches latency. The host is able to control and then eradicate the pathogen. For this reason, when attempting to make a live-attenuated vaccine in our lab, we took *M. tuberculosis* and made one of its key genes for surviving during latency non-functional. Previous researchers in my lab tested a strain of *M. tuberculosis* in which they had made this gene, called *mosR*, non-functional. The mutated strain, called H37RvΔ*mosR,* was less harmful when tested in mice and caused immune responses that could protect against future *M. tuberculosis* infection (12, 13).

I then tested our live-attenuated vaccine in mice. It performed well in terms of causing an immune response that could be protective in the future. We compared it to the immune response that the current vaccine, BCG, generated and found that the live-attenuated vaccine performed better. However, despite earlier testing indicating that this live-attenuated vaccine would be safe, I discovered that it caused tissue damage and was able to continue growing within the host. These results inspired us to take a closer look at the gene, *mosR*.

When originally testing what happens to *M. tuberculosis* without *mosR*, my lab colleagues used a strain of *M. tuberculosis* known for being a weak producer of a lipid known as PDIM. Just like individual humans have different appearances and characteristics despite our DNA being 99.9% the same, *M. tuberculosis* has different strains that have different characteristics too despite being very similar overall. The lipid PDIM is part of the cell wall of *M. tuberculosis* and can protect it from cell wall stressors, like detergents, that can break apart and kill bacteria. Additionally, when there are a lot of *M. tuberculosis* bacterium living in one cell, they express PDIM as a protein that can help break open the host cell and release them. In my study of the *mosR* in this thesis, I used a strain of *M. tuberculosis* that better produces PDIM to see if this factored into its ability to survive and cause disease in the host.

In an *M. tuberculosis* strain called "CDC1551", my lab once again made *mosR* non-functional. This mutant strain, called CDCΔ*mosR* survived in the host and caused damage similar to if it still had *mosR*. I also saw that the new mutant was more resistant to chemicals that attack the cell wall than the prior mutant. Since PDIM protects against these chemicals, I thought this could mean that PDIM helped one strain to survive better than the other.

To confirm this more fully, I looked at whether the *mosR* protein could possibly be a part of controlling PDIM. PDIM is made from a group of translated proteins and blocking the gene

expression of that group results in no PDIM production. Bacteria have mechanisms for controlling expression, one method occurs when some proteins are able to attach to DNA and prevent those genes from being expressed as proteins. My colleagues previously had shown that *mosR* could bind to certain DNA. Using a predictive pattern, I was able to show it theoretically could attach itself to the area that expresses genes needed for PDIM (Figure 3b-c). While this finding was exciting, I still have to prove that *mosR* is able to do this experimentally.

To return to the idea of homeostasis, in this section of my thesis I attempted to make a live-attenuated vaccine by disrupting *M. tuberculosis'* ability to survive in the latent stage. Interestingly, I found through our additional studies of *mosR* that PDIM, a key part of *M. tuberculosis*, is connected to *mosR* regulation as well. In all, *M. tuberculosis* is able to still persist through to that homeostasis in latency even without one of its key genes- indicating that it has many overlapping tools we need to continue studying.

**Superinfection**

I disrupted *mosR* to see how changes in *M. tuberculosis* could disrupt homeostasis, I also wanted to see how outside disruptions can hinder host control of the bacteria. For example, people who have latency can undergo reactivation if they get another disease that affects their body's ability to control *M. tuberculosis*. One example of this is human immunodeficiency virus (HIV) infection, which increases the likelihood of *M. tuberculosis* reactivation by 20x (14).

When SARS-CoV-2 began causing illness in 2019 and 2020, I decided to test how SARS-CoV-2 (SCV2) infection might affect an *M. tuberculosis* infection. I, with the help of my lab colleagues, did this by first infecting mice with *M. tuberculosis* and then infecting them with SARS-CoV-2 at different stages of the disease. I then looked to see if there were more *M. tuberculosis* bacteria throughout the mice organs as a result, and if there were changes to the

granuloma structures. I found that there were slight increases in the number of bacteria in the lungs and even greater increases in the spleen. I also found that the mice infected with TB/SCV2 had less overall tissue damage than those with just TB, but the granuloma-like structures seemed similar to a regular TB infection.

I and my colleagues then looked at the host immune response. Immune cells use certain signals, called cytokines, to communicate with each other. Some cytokines signal for more inflammation (pro-inflammatory) while others balance the inflammation (called anti-inflammatory). Pro-inflammatory cytokines, also known as type 1, signal for more bacteria-killing cells, and are associated with granulomas maintaining their structure. When we looked at these immune signals in the mice, we found that cytokines associated with mycobacterial control, type 1, were lower than expected and those that were associated with mechanisms of reactivation (type 2) were higher. These changes were not large but followed a similar pattern to our previous data.

Finally, I also looked at how *M. tuberculosis* reacted to the change of SCV2 infection. Just like *M. tuberculosis* expresses different genes as it becomes trapped by the granuloma, we wanted to see if it also did so when its host became infected with SCV2. I looked specifically for genes expressed when disease is active and when it's latent. *M. tuberculosis* impacted by SCV2 increased the expression of genes associated with active disease. I took this to mean that the way SCV2 impacted the immune system also resulted in impacts large enough to be detectable for *M. tuberculosis.* In conclusion, the data suggests that SCV2 infection impacts the homeostasis between host and pathogen. It does this through slight immune changes, creating a loss of control allowing bacteria to spread (Figure 4) (15).

Although this is where the research done in my thesis concludes, *M. tuberculosis* remains a global health threat and continuing research should be done. For *mosR*, studies examining how fast *mosR* mutants grow, the genes they express and if *mosR* binds to the PDIM gene experimentally will be important future directions. The data above will shed light on other genes *mosR* may impact and increase our understanding of latency. For the coinfection study, examining how different strains of COVID-19 and *M. tuberculosis* impact the results of dual-infection could help determine future treatment protocols and increased knowledge for future pandemics.

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# LIST OF FIGURES

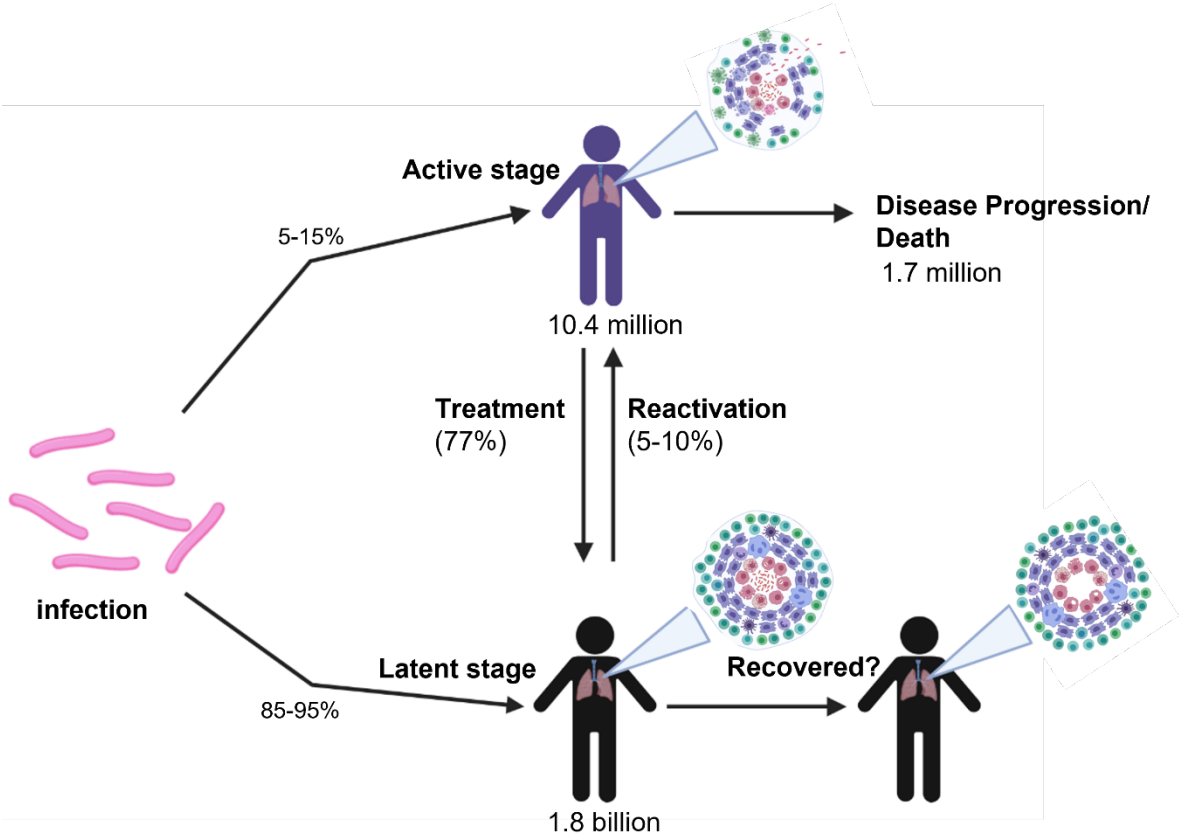


**Figure 1. Depiction of TB disease.** After infection 5-15% of people progress to an active stage, while 85-95% of people progress to latency. People with active disease can move to latency via treatment about 77% of the time. About 5-10% of people with latency are likely to reactivate back towards active disease. Each disease state also has a granuloma (spherical mass with small rods depicting *M. tuberculosis* inside). During active state or reactivation, a granuloma may not be able to contain the *M. tuberculosis.* While in the latent stage, the bacteria are contained within.

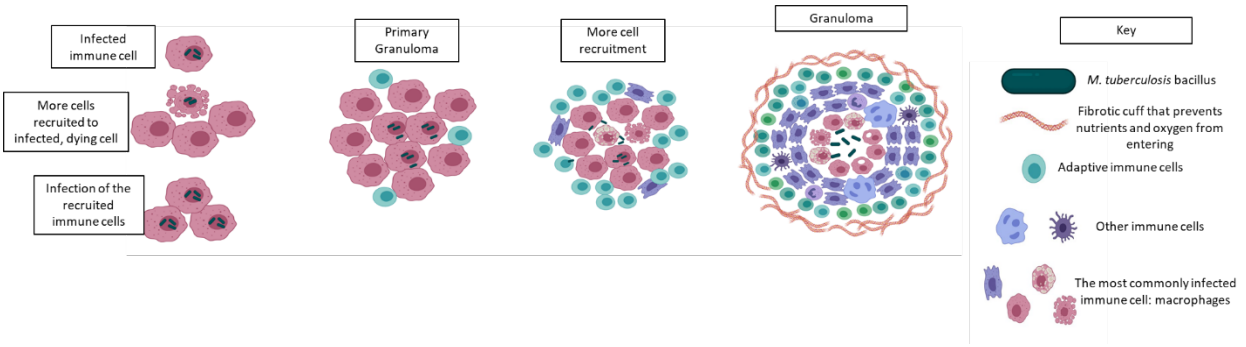


**Figure 2. Granuloma Formation for TB.** *M. tuberculosis* will replicate within an infected macrophage. This immune cell will then die and other macrophages will surround it. The *M. tuberculosis* is then able to infect these as well. This occurs repeatedly forming a mass of cells surrounding the infected center. This mass, through the death and recruitment of more host cells and growing pressure resulting from size, forms a cuff along the outside of the tissue lesion. This cuff prevents nutrients and oxygen from getting in.

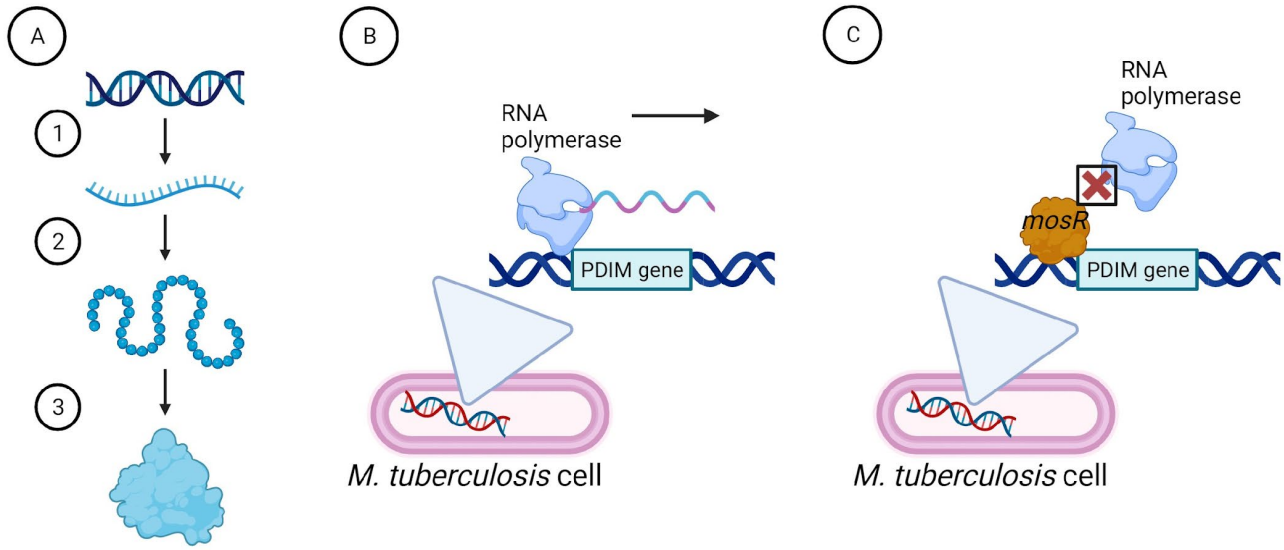


**Figure 3. Gene expression.** A) For gene expression to occur, first (1) the DNA strand is read by a protein into a transcript called mRNA. Then, this transcript is translated into a string of amino acids (2), the building blocks of proteins. Once translation is done, the string of amino acids folds into the protein the cell uses (3). B) Schematic of how PDIM expression begins without *mosR* expression. C) Theoretical consequences of *mosR* expression. *mosR* is able to bind to the area that the transcribing protein, RNA polymerase, needs to bind to in order to express PDIM. This prevents PDIM genes from being expressed.

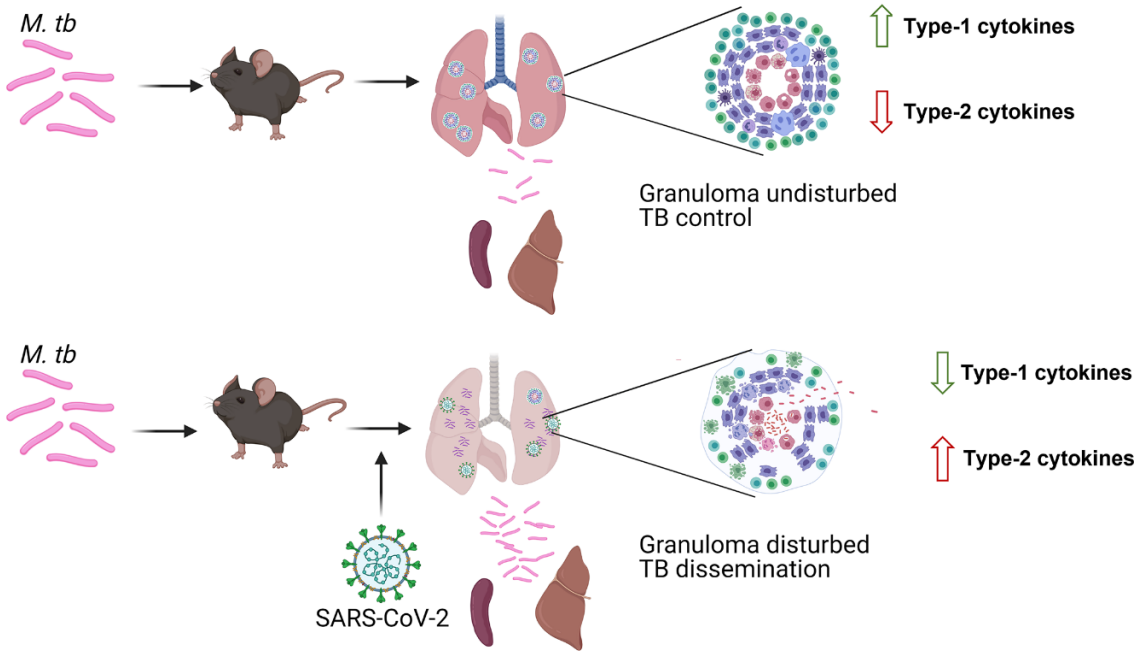


**Figure 4. Graphical Hypothesis of the TB/SCV2 coinfection.** First, mice are infected with *M. tuberculosis*. They are then dually infected with SCV2. This results in disturbances in the immune system resulting in a decrease in type 1 and increase in type 2 immune signals. Under these conditions, host control of *M. tuberculosis* is disturbed and the bacteria are able to spread both within the lung and to other tissues. Image from (Hildebrand, R.E. et. al., 2022, Fig. 10)